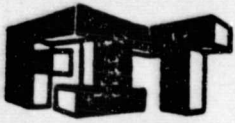


General Disclaimer

One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.
- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.
- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.
- This document is paginated as submitted by the original source.
- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

CR 160144



Florida Institute of Technology

Melbourne, Florida 32901

PHOTOSYNTHETIC CARBON REDUCTION
BY SEAGRASSES EXPOSED
TO ULTRAVIOLET B RADIATION
FINAL TECHNICAL REPORT

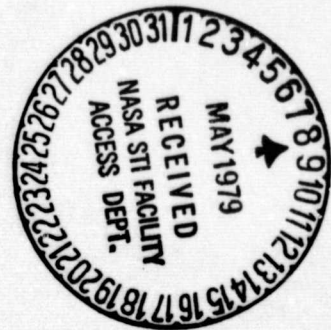
(NASA-CR-160144) PHOTOSYNTHETIC CARBON
REDUCTION BY SEAGRASSES EXPOSED TO
ULTRAVIOLET B RADIATION Final Technical
Report (Florida Inst. of Tech.) 45 p HC
A03/MF A01

N79-21742

Unclas
14752

CSCS 06C G3/51

School of
Science and
Engineering



Submitted to
National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
Technical Library Branch
Attention: Retha Shirkey JM6
Houston, TX 77058

Contract No. NAS9-15516

Photosynthetic Carbon Reduction
by Seagrasses Exposed
to Ultraviolet-B Radiation

Final Technical Report

Submitted by
Florida Institute of Technology
Melbourne, Florida 32901
(305) 723-3701

15 March 1979

Table of Contents

	Page
Forward	ii
I. Introduction	1
II. Methods and Materials	
A) Seagrass Collection and Saline Analysis	5
B) Photosynthesis	5
C) Ultraviolet and Visible Irradiation	9
D) <u>In Situ</u> Studies	11
E) Epiphyte Studies	11
F) Data Analysis	13
III. Results and Discussion	
A) Preliminary Studies	15
B) Ultraviolet-B Studies	18
C) Photorepair Studies	23
D) The UV-A Effect	26
E) <u>In Situ</u> Experiments	32
F) Epiphyte Studies	34
IV. Conclusions	37
V. Literature Cited	39

Foreword

This investigation was conducted in the Biological Sciences Department of Florida Institute of Technology, under the direction of Dr. Gary N. Wells. The program was funded by the Medical Sciences Division, with Dr. D. S. Nachtwey providing program direction.

While all members of the Florida Institute of Technology project team contributed to all portions of the study through frequent meetings to assure good coordination, the primary responsibility for Phase I and Phase II investigations belong to Mr. Robert Trocine and Mr. John D. Rice. Others who provided technical assistance include Mr. Bill Aspden and Mrs. Kathy Austin.

The authors acknowledge the support throughout the program of Dr. G. C. Webster, Head of the Department of Biological Sciences, Mrs. Carolyn Sorrell, Departmental Secretary, and Miss Dee Dee Looney for typing the report manuscript.

Introduction

A consequence of the reduction in the stratospheric ozone layer caused by air pollutants (fluorocarbon aerosols and NO_x from jet exhausts for example) is an increase in the amount of penetrant ultraviolet radiation in wavelengths between 280 and 315 nm (UV-B). The impact of this increase in UV-B upon the biosphere is not fully defined, and has been the subject of considerable investigation in recent years (9). An understanding of the potential environmental hazards from UV-B becomes increasingly important as air travel steadily increases and the implementation of the space shuttle program approaches. The primary thrust of studies designed to assess the biological impact of UV-B has been in terrestrial and freshwater biosystems (2, 7). Studies in the marine environment have thus far been limited to simple algal systems (5, 6) and aquatic microorganisms (4, 8).

The intent of this research effort has been to study the effects of UV-B on a more complex marine system, that of seagrasses. These angiosperms are of considerable ecological importance in shallow water marine and estuarine systems in terms of total productivity and diversity. Many marine organisms utilize seagrasses as a spawning area and/or site of egg deposition. Other species may profit from the seagrasses as a source of food or protection from predators. A significant reduction in abundance of seagrasses, or their relocation to deeper waters may have a profound effect on many estuarine trophic levels. Three species of seagrasses were selected for this study on the basis of their dominance in the system, contribution to total productivity, and importance to the

life histories of organisms in the Indian River lagoonal system along the central Florida east coast. They were Halophila engelmannii, Halodule wrightii, and Syringodium filiforme (10). These seagrasses form an excellent experimental system as their areas of dominance fall more or less along a natural gradient of UV-B and photosynthetically active radiation (PAR) penetration (fig. 1).

Photosynthesis is a necessary process for the survival of all plant forms. Therefore, the effect(s) of UV-B upon photosynthesis in seagrasses is a logical measurement of their physiological response to this radiation. The major body of UV-B research on photosynthesis has involved terrestrial plants. This data may be used for purposes of comparison, for while there are morphological differences between terrestrial and marine plant forms, basic photosynthetic mechanisms appear similar in most respects. Ultraviolet radiation in general has been shown to be a powerful inhibitor of photosynthesis and associated reactions. These include the Hill reaction, non-cyclic photophosphorylation, and photo-reduction (5). In particular, UV-B has been shown to cause a significant and cumulative reduction of photosynthesis in Rumex patientia (11). After extended irradiation an actual decrease in leaf area and dry weight was observed. Pisum sativum when exposed to UV-B has also responded with a decrease in net photosynthesis (3). Inhibition of electron transport and the Hill reaction, as well as swelling of thylakoid membranes were recorded. The work of Thai and Garrard (15) and Basiouny et al. (1) have shown both C_3 and C_4 photosynthesis to be sensitive to UV-B. Studies by Wells and Nachtwey (16) have demonstrated the sensitivity of photosynthesis by Ruppia maritima to UV-B irradiation. The sensitivity of Ruppia establishes at least an empirical similarity between the response of terrestrial

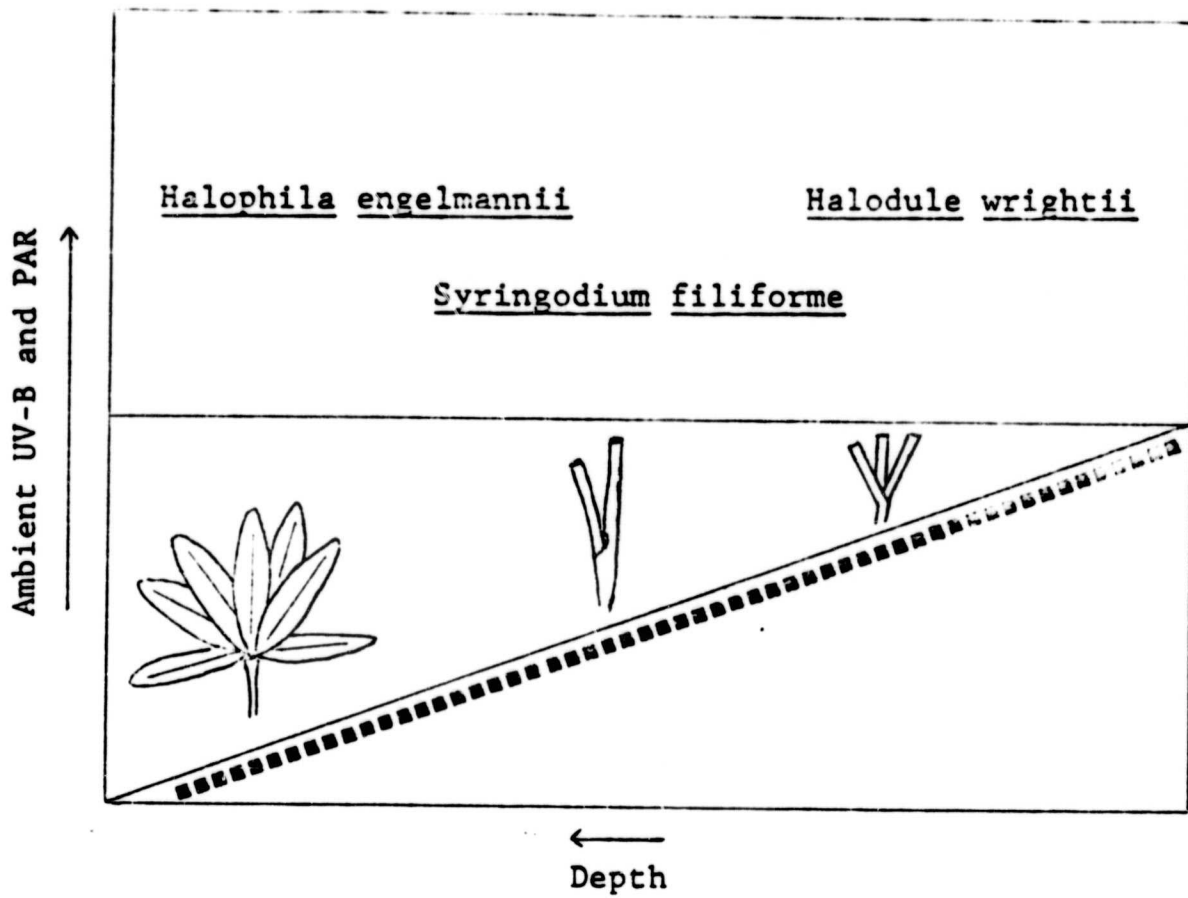


Figure 1. Seagrass dominance as a function of depth and radiation intensities.

plants and seagrasses to UV-B.

The actual mechanism(s) by which UV-B inhibits photosynthesis is not known. The purpose of this study is not to investigate this mechanism, but rather to determine the sensitivity of photosynthesis in the seagrasses Halophila, Halodule, and Syringodium, and monitor their photosynthetic response to levels of UV-B simulating atmospheric ozone depletion. Four basic questions were to be answered:

- A) Does the seagrass possess a photosynthetic tolerance for UV-B?
- B) If so, is the seagrass already existing at this level in the environment? This question was investigated by complimentary use of in vitro and in situ experimentation.
- C) If ambient UV-B is the maximum the seagrass can tolerate without photosynthetic inhibition, what quantitative effects will a further increase in UV-B induce?
- D) If the seagrass is not currently experiencing its maximum tolerable UV-B level, how much more can it tolerate?

Further experiments explore the possible attenuation or repair of UV-B induced photosynthetic inhibition by PAR, the role of epiphytic growth upon seagrasses as a protective UV-B shield, and finally the inhibition of photosynthesis in response to UV-A is studied.

Methods and Materials

A) Seagrass collection and Saline analysis.

Seagrass samples were collected intact from established sample sites (fig. 2), and transported to the laboratory the morning of each experiment. Water, also obtained fresh for every experiment, was filter sterilized through a Buchner funnel with #4 Whatman paper, followed by Millipore filtration (0.45μ). Total alkalinity, carbonate alkalinity, total CO_2 (all forms), $[\text{HCO}_3^-]$ and $[\text{CO}_3^{=}]$ were determined as described by Strickland and Parsons (13). Using this data, available $\mu\text{g } [^{12}\text{C}]$ in seawater was calculated for the incorporation procedure (section B). Dissolved oxygen was measured using the Winkler method (14); while salinity was calculated on the basis of the refractive index obtained with an American Optical T/C Refractometer. The formula to convert refractive index to salinity is:

$$\text{Salinity (ppt)} = (\text{R.I.} - 1.3330) \times 0.54 \times 10,000$$

Once the seawater was prepared, samples of leaf tissue were excised, cleaned of epiphytes, their fresh weights determined and placed in petri plates containing filter sterilized seawater (FSSW) to be exposed to the experimental irradiation.

B) Photosynthesis.

Incorporation of $[^{14}\text{C}]$ sodium bicarbonate into acid-stable intermediates was used to determine photosynthetic rates following exposure to UV-B and/or PAR. After irradiation, leaf tissue was placed in 100 ml beakers containing 20 ml FSSW and equilibrated at $700 \mu\text{E}/\text{m}^2/\text{sec}$ and 30°C for 10 minutes in a water-cooled incorporation chamber similar to

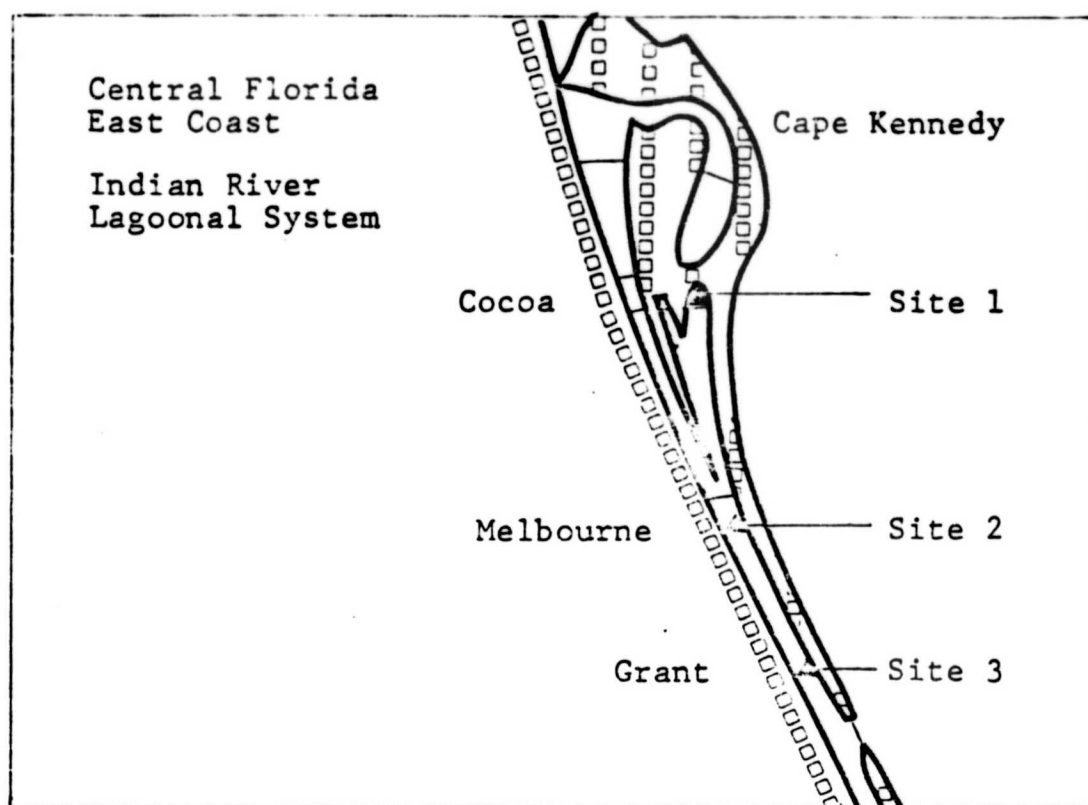


Figure 2. Test area and sampling sites; Indian River lagoonal system.

figure 3. After equilibration, the leaf tissues were transferred to 100 ml beakers containing 5 ml fresh FSSW, returned to the chamber, and 15 μ l of [14 C] sodium bicarbonate (1 mCi/ml, 50 mCi/mmol) was added to each of the samples. Following an incorporation period of 15 minutes the leaf tissue was removed, washed thoroughly with deionized water (D.I. water) and homogenized in glass Ten-Broeck homogenizers containing 1 ml of hot methanol. The homogenates were clarified by centrifugation at 2300 RPM for 5 minutes in 15 ml conical tubes and methanol soluble fractions (MSF) transferred to 35 ml conical tubes; methanol insoluble pellets were washed 3 successive times by suspending in 1 ml of hot methanol and clarifying by centrifugation. The hot methanol washes were pooled with the MSF's and the pellets resuspended in 1 ml of D.I. water, covered with parafilm, and allowed to extract for 12 hours at room temperature.

Chlorophyll was extracted from the MSF using the ratio of MSF: ether: D.I. water (1:1:1.2). The upper ether layer was removed from the methanol-water fraction and brought to 10 ml with anhydrous ethyl ether and total chlorophyll determined according to the method of Strain and Svec (12) using the equation:

$$\mu\text{g Chl.} = 7.12 (A_{660}) - 16.8 (A_{642.5}) \times 10$$

After extraction for 12 hours in water, the methanol insoluble pellets were resuspended and clarified at 2300 RPM for 3 minutes and the supernatant fraction retained. The pellets were washed twice more with 1 ml of D.I. water and all the water supernates combined with the methanol-water fractions and total volumes recorded. A 0.1 ml aliquot of the methanol-water fraction was added to 10 ml of Aquasol-2 (New England

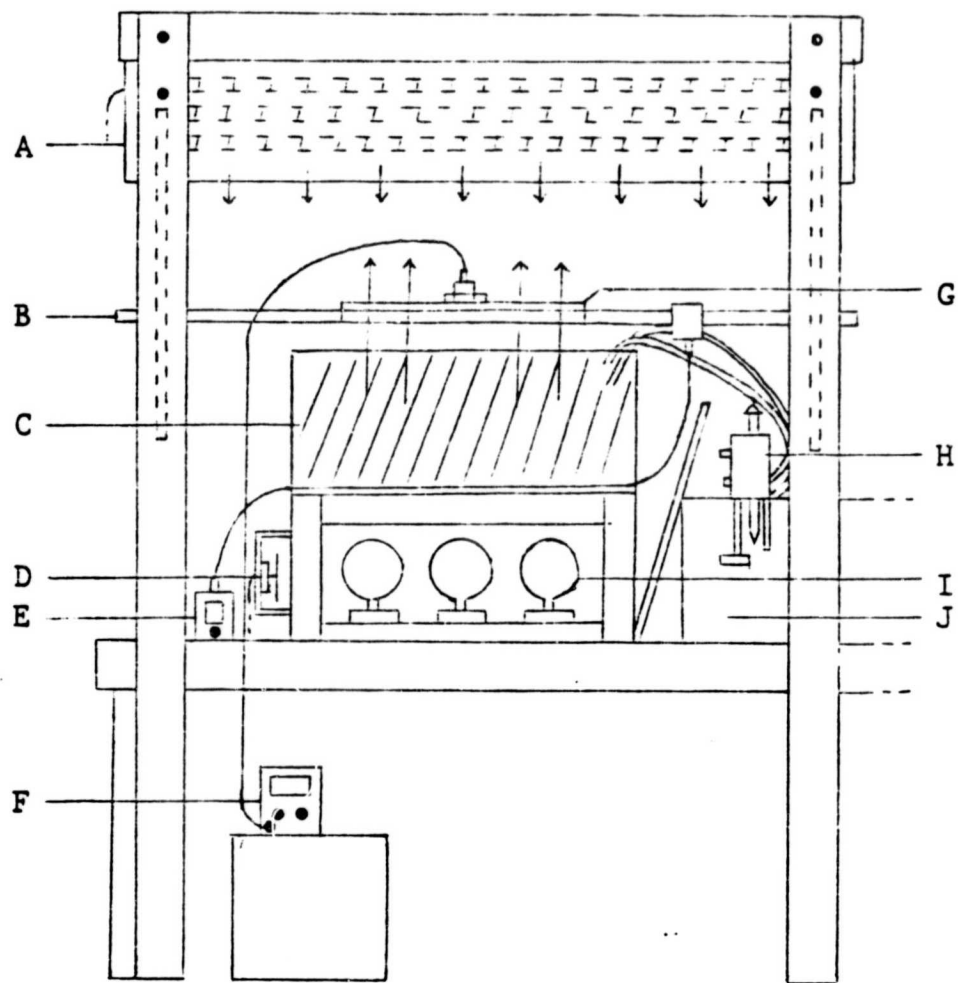


Figure 3. Ultraviolet and PAR irradiation apparatus; A) FS-40 fluorescent sun lamps; B) adjustable test platform; C) heat sink; D) fan; E) Sunburn Ultraviolet Meter; F) LICOR photometer; G) plexiglass window; H) water pump; I) PAR light bank; J) cooling tank.

Nuclear) liquid scintillation cocktail and counted for radioactivity in a Beckman LS 100-C scintillation counter. Counting efficiency was determined to be 72 percent. The equation to calculate the photosynthetic rate is:

$$\mu\text{g C/mg Chl/hr} = \frac{\text{DPM}_{\text{fixed}}}{\text{DPM}_{\text{added}}} \times 1.06 \times \frac{\mu\text{g }^{12}\text{C}}{\mu\text{g Chl}} \times 4000$$

C) Ultraviolet and Visible irradiation.

Ultraviolet-B radiation was provided in the laboratory by a bank of 6 Westinghouse FS-40 fluorescent sun lamps (fig. 3). Dose rates of 3 CPM to 23 CPM were achieved by selectively adjusting the lamp to tissue distance. Because there was a slight but steady decrease in ultraviolet radiation output by the FS-40 sun lamps for 40 to 50 minutes before they stabilized, the lamps were turned on approximately 60 minutes prior to the start of each experiment. After being stripped of epiphytes, leaf tissues to be irradiated were placed in petri plates containing FSSW and set beneath the "sun lamps". A black, absorptive background was used to prevent re-radiation from the test platform and altering of the UV-B dose rate and total dosage. Tissue samples to be irradiated with UV-B were placed beneath a layer of Kodacel (5 mil, Eastman Kodak) which filters out UV-C (40-280 nm) produced by the FS-40 sun lamps, but allows UV-A (315-400 nm) and UV-B (280-315 nm) transmission. Irradiation controls were positioned underneath a sheet of Mylar (10 mil, DuPont) which filters out UV-B and UV-C, allowing UV-A to be transmitted. Dark controls were prepared with each experiment. These received no irradiation of any sort, and served as a monitor of system integrity.

The UV-B received by the leaf tissue was measured using a Sunburn Ultraviolet Meter (Solar Light Company) with a remote probe which was fixed in the test platform at the level of the samples. A film of Kodacel was placed over the probe to insure accurate measurement of the UV-B received by the test samples. All Kodacel and Mylar films were "burnt in" for approximately 100 hours prior to experimental use. Each set of films were aged to the same degree and replaced after 50 hours of use to minimize changes in spectral transmission.

Field measurements have established an UV-B dose rate of 6 CPM as the maximum experienced by Halodule and Syringodium with any regularity. Total dosage rarely exceeds 2000 counts for Halodule and only 1500 counts for Syringodium at mean leaf depth. Halophila is common at increased depths relative to the other seagrasses and experiences a dose rate of 6 CPM only at the upper limit of its range; total dosage received is normally less than 1000 counts during a daily cycle. These dose rates and dosages set the baseline for studying the tolerance of the seagrasses to increased UV-B radiation.

To determine if a photorepair mechanism was operative which would be active in reversing or attenuating UV-B induced photosynthetic damage, a clear plexiglass plate was incorporated into the test platform and a bank of six 300 watt Westinghouse light bulbs placed beneath it to provide PAR backlighting while UV-B was received from above. Light intensities both in the field and the laboratory were measured with a LICOR quantum/radiometer/photometer (model LI-185A). Following irradiation, the tissues were placed in the dark to avoid extraneous light effects until the photosynthetic rate could be measured (normally within 30 minutes of irradiation).

D) In situ studies.

Special submersible incorporation chambers were designed to allow $[^{14}\text{C}]$ assimilation in the field without danger of contaminating the environment (fig. 4). The chambers were placed at the characteristic depth for each seagrass at a test site. Ambient UV-B and PAR intensities were measured every half hour at the air/water interface and at the level of the chambers for the duration of the experiment (usually 10 AM to 4 PM). Samples of the seagrass were prepared for each experiment as described in section A, with the exception that the samples were placed in plastic vials containing an excess of FSSW for transportation to the test site. At the site the samples were placed into the incorporation chambers, one screened with Mylar, the other two covered with Kodacel. The sets of chambers were submerged at two hour intervals (for example 10 AM, noon, and 2 PM). Water in each chamber is changed every two hours to reduce the risk of oxygen toxicity influencing the results. At the end of the test period all chambers were injected with 15 μl (1 $\mu\text{Ci}/\mu\text{l}$) of $[^{14}\text{C}]$ sodium bicarbonate and resubmerged for 15 minutes to incorporate, after which the chambers were removed from the system, broken down (taking care to avoid contaminating the area) and tissues washed with D.I. water. The samples were immediately placed in vials containing 3 ml of methanol to kill them and prevent $[^{14}\text{C}]$ loss. Chlorophyll extraction and isolation of acid-stable intermediates were performed in the laboratory as described in section B.

E) Epiphyte studies.

Samples of the seagrass to be tested were collected the morning of the experiment and prepared as in section A with the exception of

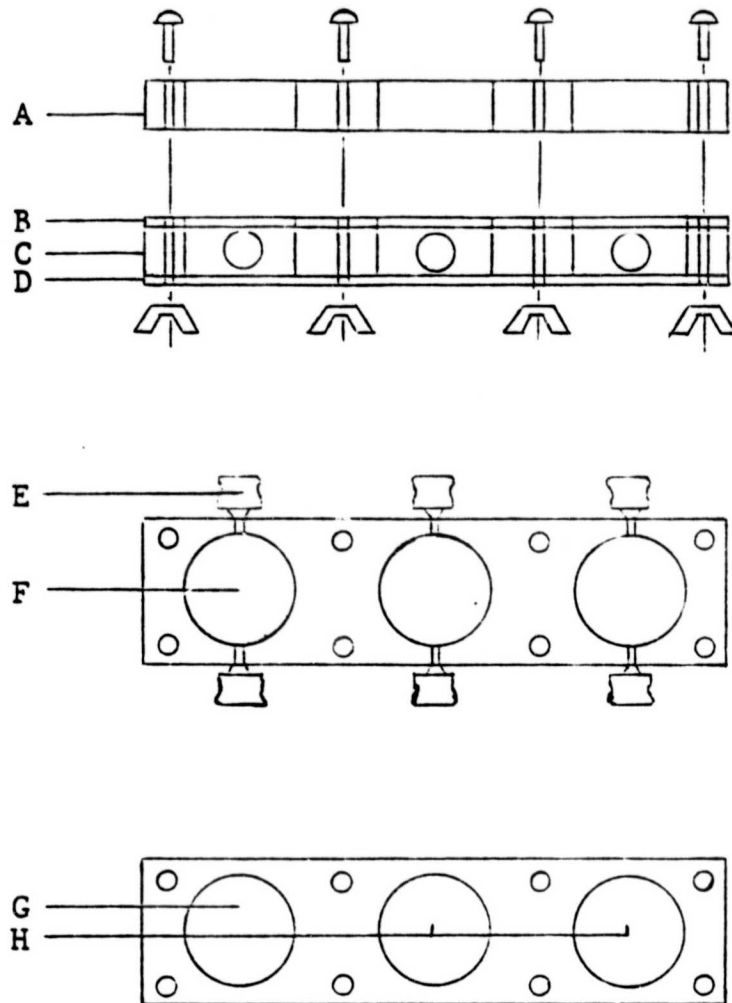


Figure 4. In situ incorporation chambers; A) top plate; B) rubber gasket; C) bottom plate; D) plexiglass butt plate; E) injection port; F) incorporation well; G) Mylar window; H) Kodacel windows.

epiphyte removal. Three samples were weighed with epiphytes intact and placed in petri plates containing FSSW for UV-B irradiation. A fourth sample was weighed with epiphytes intact and again after the epiphytes were removed to determine the amount of epiphytic cover. One of the three remaining samples was placed beneath a Mylar filter while the others were screened with Kodacel. After exposure to UV-B the epiphytes were removed and the photosynthetic rate determined (section B).

F) Data analysis.

Initially it was felt the most satisfactory method of analysing the data from each series of experiments was on the basis of the photosynthetic rate ($\mu\text{g C fixed/mg Chl/hr}$). However, as the study progressed it became apparent that the photosynthetic rate of each seagrass fluctuates greatly throughout the year (fig. 5). To remove this variation from consideration the results were expressed as percent inhibition of photosynthesis (see equation below).

$$\% \text{ photosynthetic inhibition} = 1 - \frac{\mu\text{g C/mg Chl/hr (Kodacel)}}{\mu\text{g C/mg Chl/hr (Mylar)}} \times 100$$

Zero or dark controls (leaf tissue maintained in the dark for the duration of the experiment) were used throughout the study to test the validity of the Mylar (irradiation) controls.

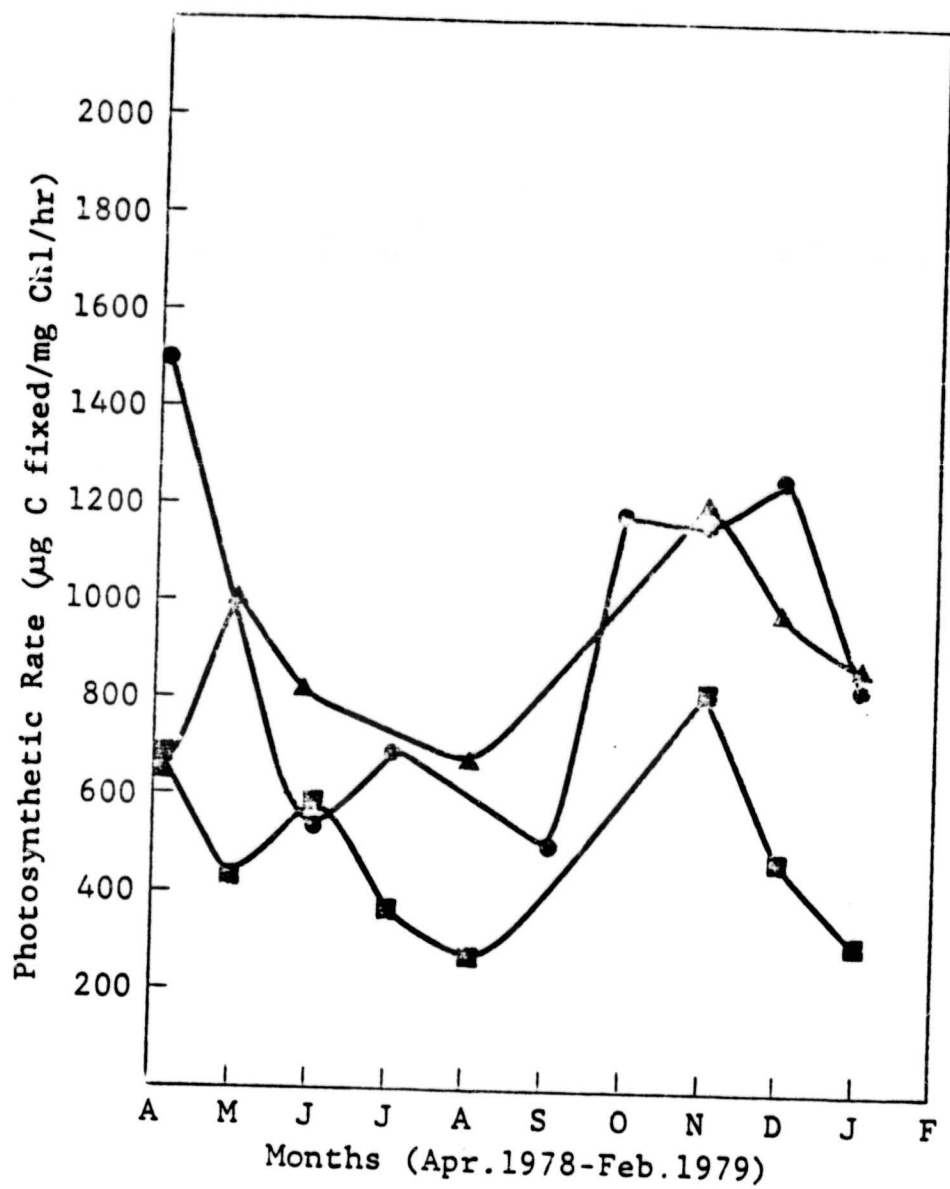


Figure 5. Seagrass photosynthetic rates during the test period; *Halophila engelmannii*, (●—●); *Halodule wrightii*, (▲—▲); *Syringodium filiforme*, (■—■).

Results and Discussion

A) Preliminary studies.

Before any experiments could be performed to characterize UV-B's effect on seagrass photosynthesis, it was first necessary to determine the photosynthetic rate of each species as a function of PAR intensity and temperature. Krepley and Wells (unpublished) have observed maximal photosynthetic rates in Halodule and Syringodium at approximately 30°C. The maximum photosynthetic rate in Halophila was found to occur at about 36°C (fig. 6). In spite of this fact an experimental temperature of 30°C was selected for all subsequent studies. The reasons for this are: One, this temperature appears optimal for photosynthesis in Halodule and Syringodium. Two, the difference in photosynthetic rates at 30°C and 36°C in Halophila is minimal and above 36°C severe thermal damage occurs in this seagrass. Once the experimental temperature was selected, studies were undertaken to monitor changes in the photosynthetic rate of each species with increasing PAR intensity at this temperature (fig. 7). Light intensities up to 850 $\mu\text{E}/\text{m}^2/\text{sec}$ were provided to samples of each seagrass without evidence of photooxidation. A PAR intensity of 700 $\mu\text{E}/\text{m}^2/\text{sec}$ was selected for all further applications of PAR. It was feared the exposure of tissues under stress to a greater PAR intensity might result in a confusion of cause and effect in later experiments. The use of 700 $\mu\text{E}/\text{m}^2/\text{sec}$ did not significantly reduce the photosynthetic rate of any seagrass when compared to higher PAR intensities.

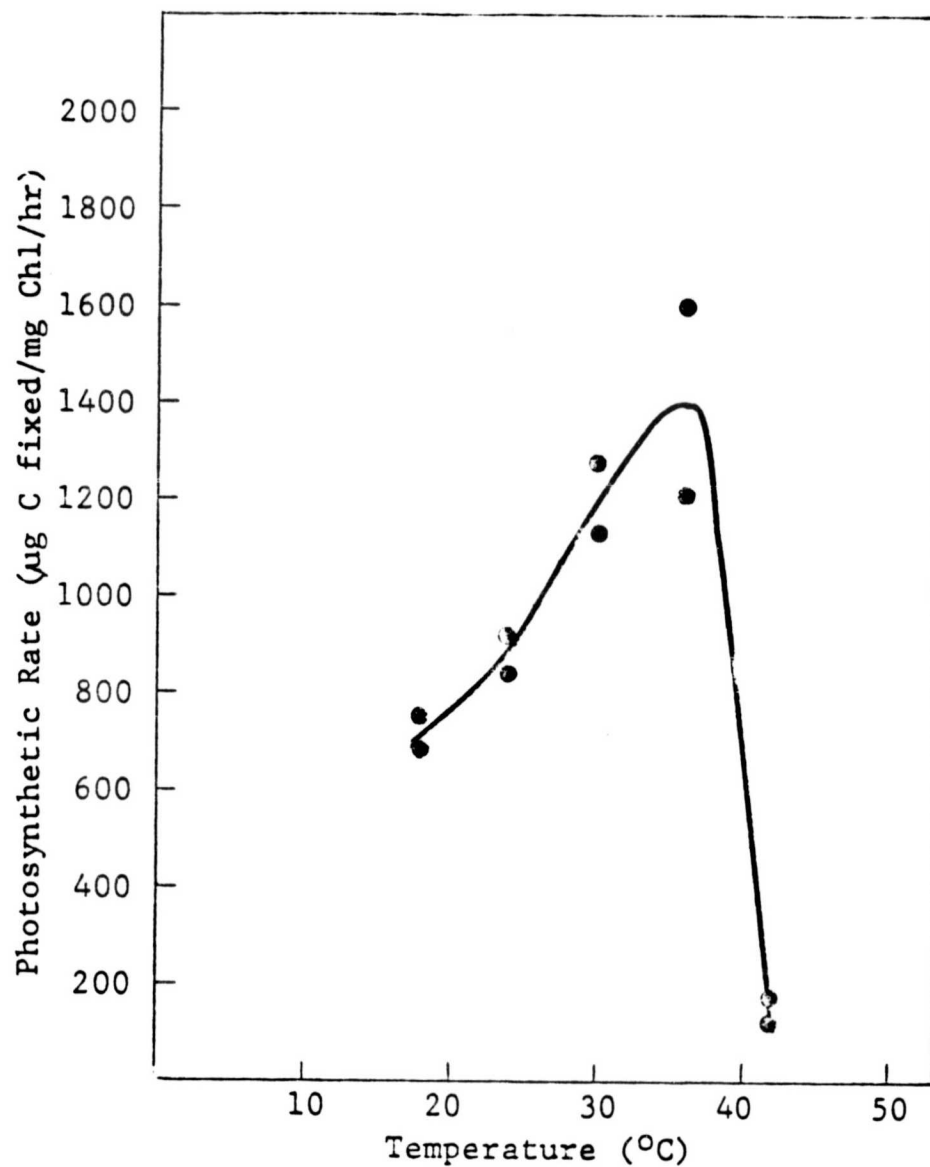


Figure 6. Photosynthetic rate of Halophila engelmannii as a function of temperature.

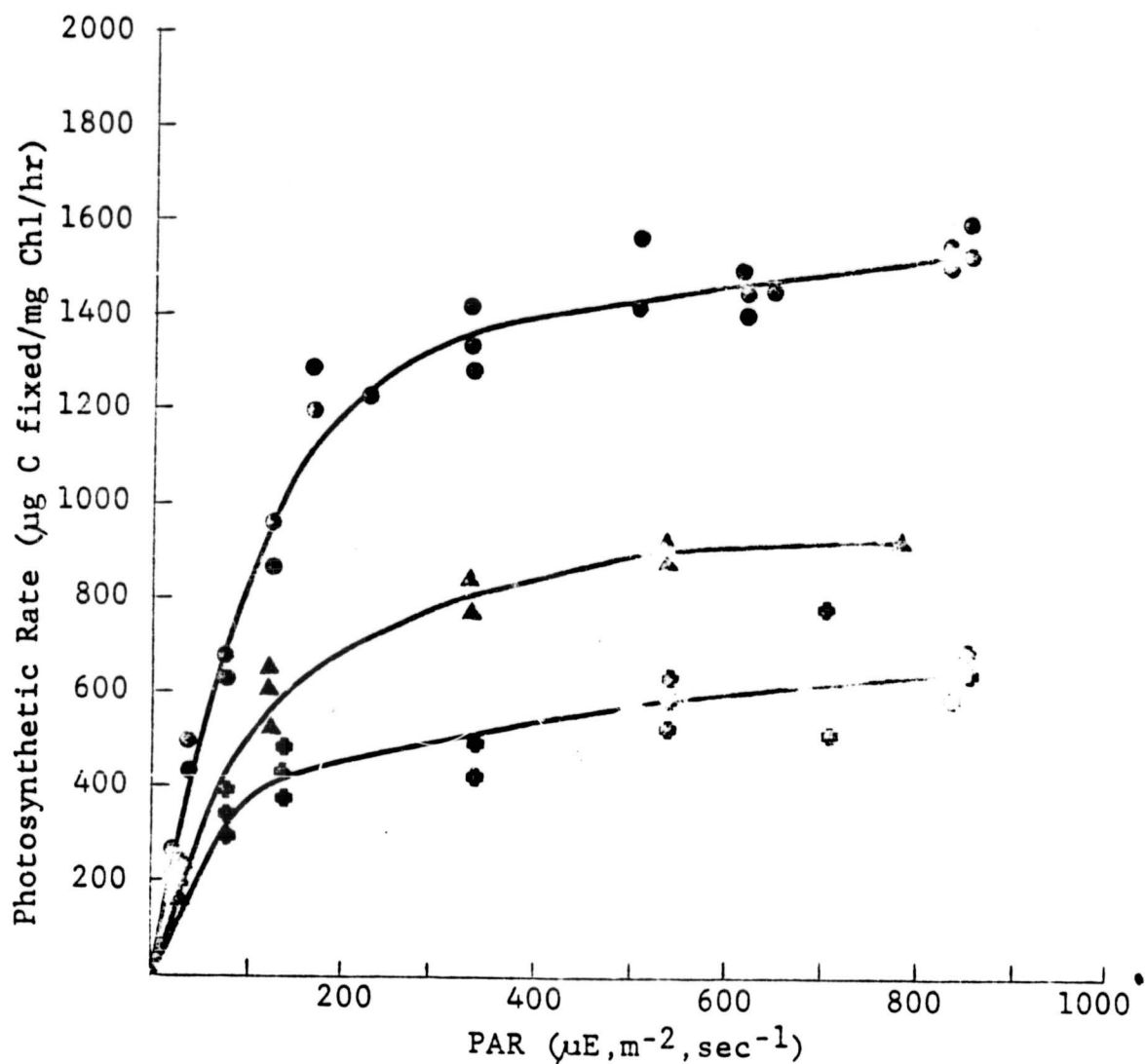


Figure 7. Photosynthetic rate as a function of PAR intensity at 30°C; *Halophila engelmannii*, (●—●); *Halodule wrightii*, (▲—▲); *Syringodium filiforme*, (○—○).

B) Ultraviolet-B studies.

The intrinsic sensitivity of seagrass photosynthesis to UV-B was determined by exposing each species to a variety of UV-B dosages and dose rates. All applications of UV-B resulted in significant photosynthetic inhibition in Halophila (fig. 8). The linear relationship between UV-B dosage and percent inhibition of photosynthesis held up to approximately 4000 counts, or 50 percent photosynthetic inhibition. Above this level of irradiation inhibition proceeds more slowly. Total UV-B dosage appears to mandate the degree of photosynthetic damage in Halophila. Results indicate this species has no tolerance to UV-B dose rates above the maximum it normally encounters (6 CPM). The lack of intrinsic tolerance to increased levels and dose rates of UV-B was not totally unexpected in Halophila. This seagrass is most abundant at depth or closer to shore in areas where turbidity is normally high. PAR intensities in these areas are low and Halophila can out compete other seagrasses with lower photosynthetic capacities. Ultraviolet-B penetration is also greatly reduced at these locations, therefore the development and maintenance of dark repair or protective mechanisms may have been unnecessary and cost prohibitive.

Halodule was also exposed to a variety of UV-B regimes. This seagrass, in contrast to Halophila, demonstrates a clear response in extent of photosynthetic inhibition to different dose rates of UV-B (fig. 9). For convenience sake, the dose rates have been grouped into three sets which were based on equivalent photosynthetic inhibition: 18-20 CPM, 12-16 CPM, and 6-8 CPM. As the dose rate increases with Halodule, the extent of photosynthetic inhibition increases. Dose rates of 6 and

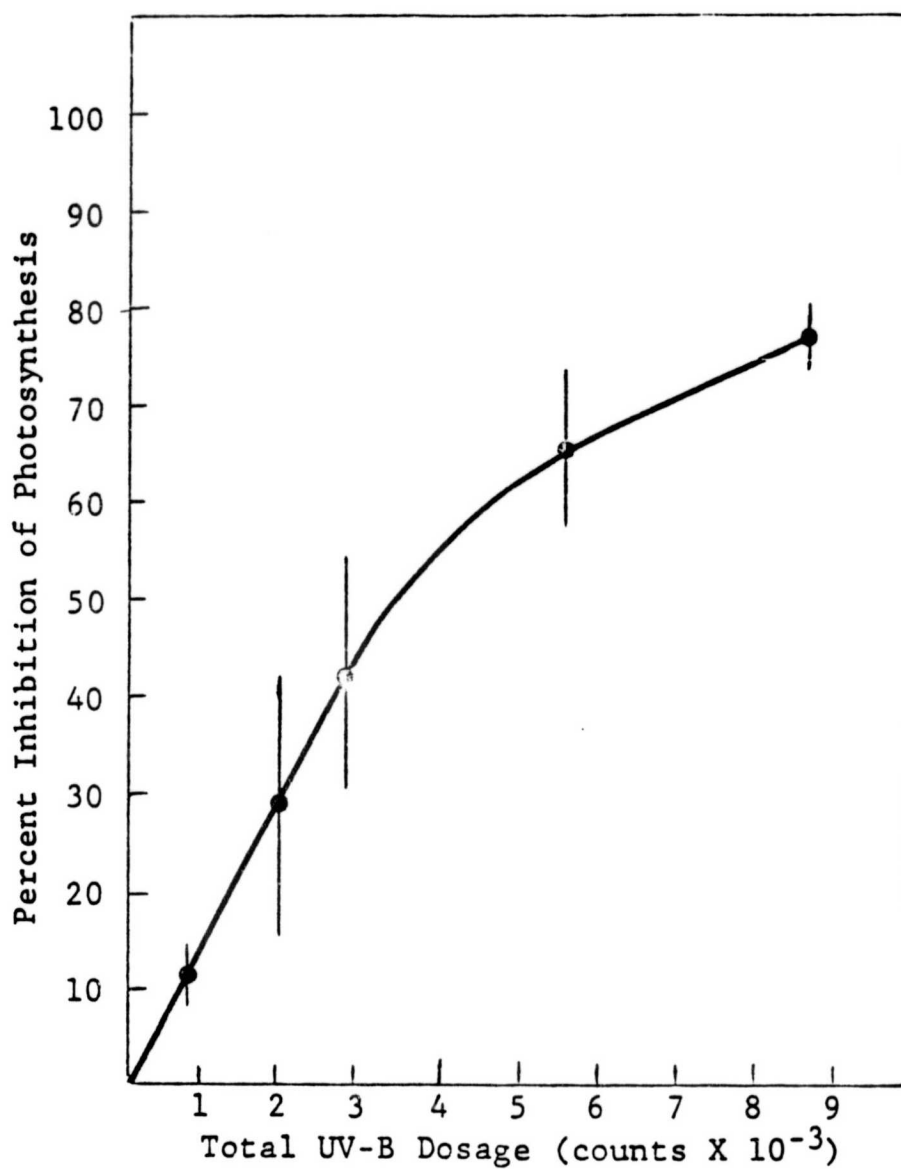


Figure 8. Inhibition of photosynthesis in Halophila engelmannii by UV-B; (vertical lines, standard deviation; closed circles, mean).

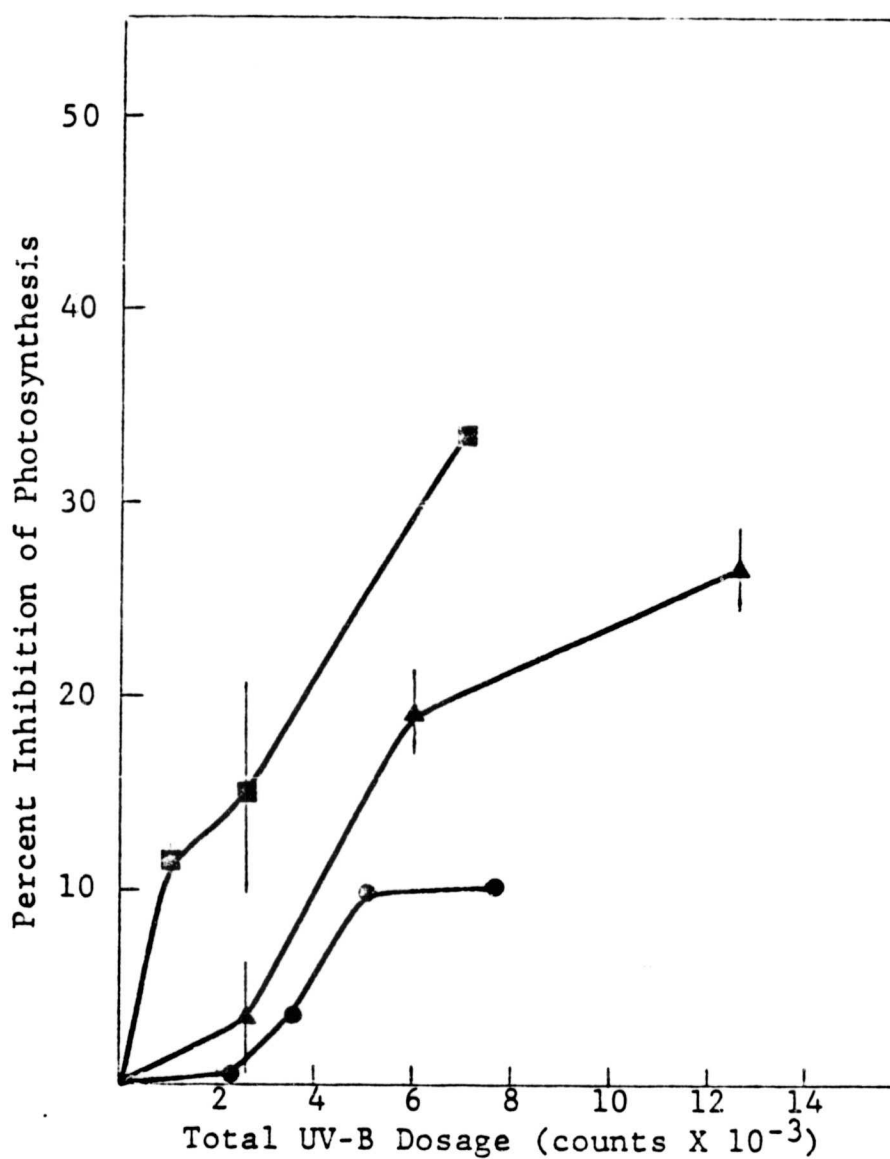


Figure 9. Inhibition of photosynthesis in Halodule wrightii by UV-B; dose rates: 18-20 CPM, (■—■); 12-16 CPM, (▲—▲); 6-8 CPM, (●—●); vertical lines, standard deviation; closed points, mean.

8 CPM were tolerated very well with less than 1 percent inhibition at a total UV-B dosage of 2500 counts. These conditions equal or exceed ambient UV-B levels found at Halodule's mean leaf depth in the Indian River during peak summer conditions. The next set of dose rates (12-16 CPM) is also tolerated well, with only 3 percent inhibition of photosynthesis at 2500 counts. The response curves for both these sets of UV-B dose rates is sigmoid in nature; 2500 counts appears to be the upper limit for intrinsic tolerance in terms of total UV-B. Above this level photosynthetic inhibition proceeds more rapidly (albeit more slowly than in Halophila). Ultraviolet-B dose rates from 18 to 20 CPM were tolerated the least, causing the most extensive photosynthetic damage. The intrinsic tolerance of Halodule to UV-B is entirely logical. Of the seagrasses in this study, Halodule experiences the greatest and most continual exposure to UV-B. Physiological adaptations to attenuate the harmful effects of UV-B would be very profitable in terms of energy utilization for this seagrass. Such adaptations, especially if passive in nature, reduce the amount of energy which must be apportioned to the repair of UV-B induced effects and allow its allocation toward other pursuits.

Syringodium required the most extensive UV-B irradiation to induce 50 percent photosynthetic inhibition, 15,000 counts, about four times that required to reach the same level of inhibition in Halophila. The photosynthetic response here, as in Halophila, showed no clear differential response to the various dose rates applied (fig. 10). The sharp, initial rise in photosynthetic inhibition indicates a lack of intrinsic tolerance to UV-B. Above 2500 total counts of UV-B the rate of inhibition increase becomes more gradual. Syringodium possesses a thick epidermal

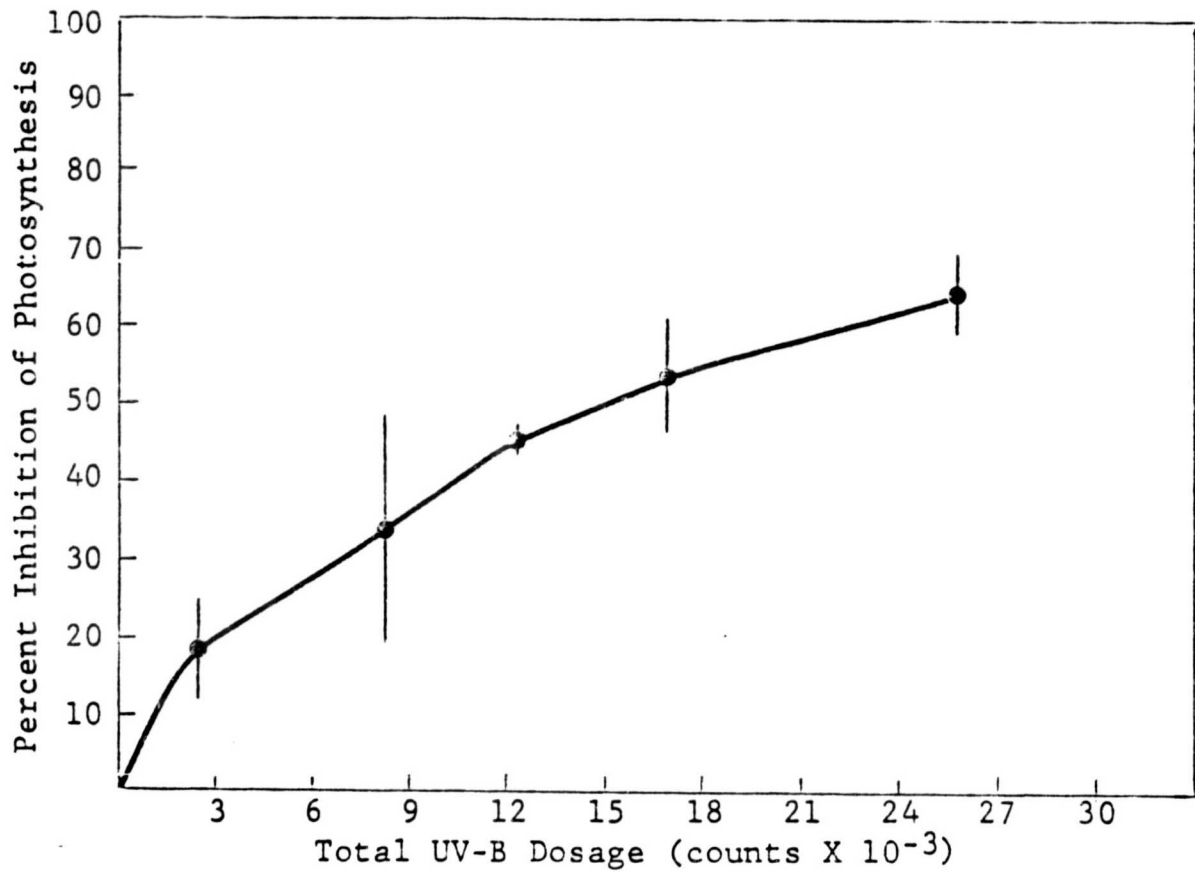


Figure 10. Inhibition of photosynthesis in Syringodium filiforme by UV-B; (vertical lines, standard deviation; closed circles, mean).

cell layer which may represent a major morphological mechanism reducing the penetration of UV-B to the more sensitive photosynthetic inner tissues, and may be responsible for the lower rate of inhibition in comparison to Halophila which lacks this thick epidermal layer. Such a design is very advantageous to Syringodium since its leaves often lie along the air/water interface. Dose rates and dosages of UV-B at this level may exceed 14 CPM (2.1 SU) or 4000 counts (fig. 11), far more than is found at the mean leaf depth.

C) Photorepair studies.

The possibility of photorepair of UV-B induced photosynthetic inhibition was examined in each of the seagrasses. Samples of leaf tissue were exposed simultaneously to UV-B and PAR. As before a variety of UV-B dose rates were used. This allowed photorepair efficiency to be studied under conditions reflecting different degrees of atmospheric ozone depletion. The UV-B dosage was initially set at 3000 counts with a PAR intensity of $700 \mu\text{E}/\text{m}^2/\text{sec}$ provided as backlighting.

In this series of experiments, Halophila failed to show any significant photorepair response to UV-B levels above those common to its environment. The presence of some mechanism is evident, but it appears to be ineffective at attenuating UV-B induced damage; 50 percent photosynthetic inhibition was again achieved at about 4000 total counts of UV-B (fig. 12). No correspondence between UV-B dose rate applied and photorepair efficiency was observed. This lack of effective photorepair was also not entirely unexpected for Halophila. To evolve and maintain a highly efficiency photorepair mechanism to a form of radiation rarely

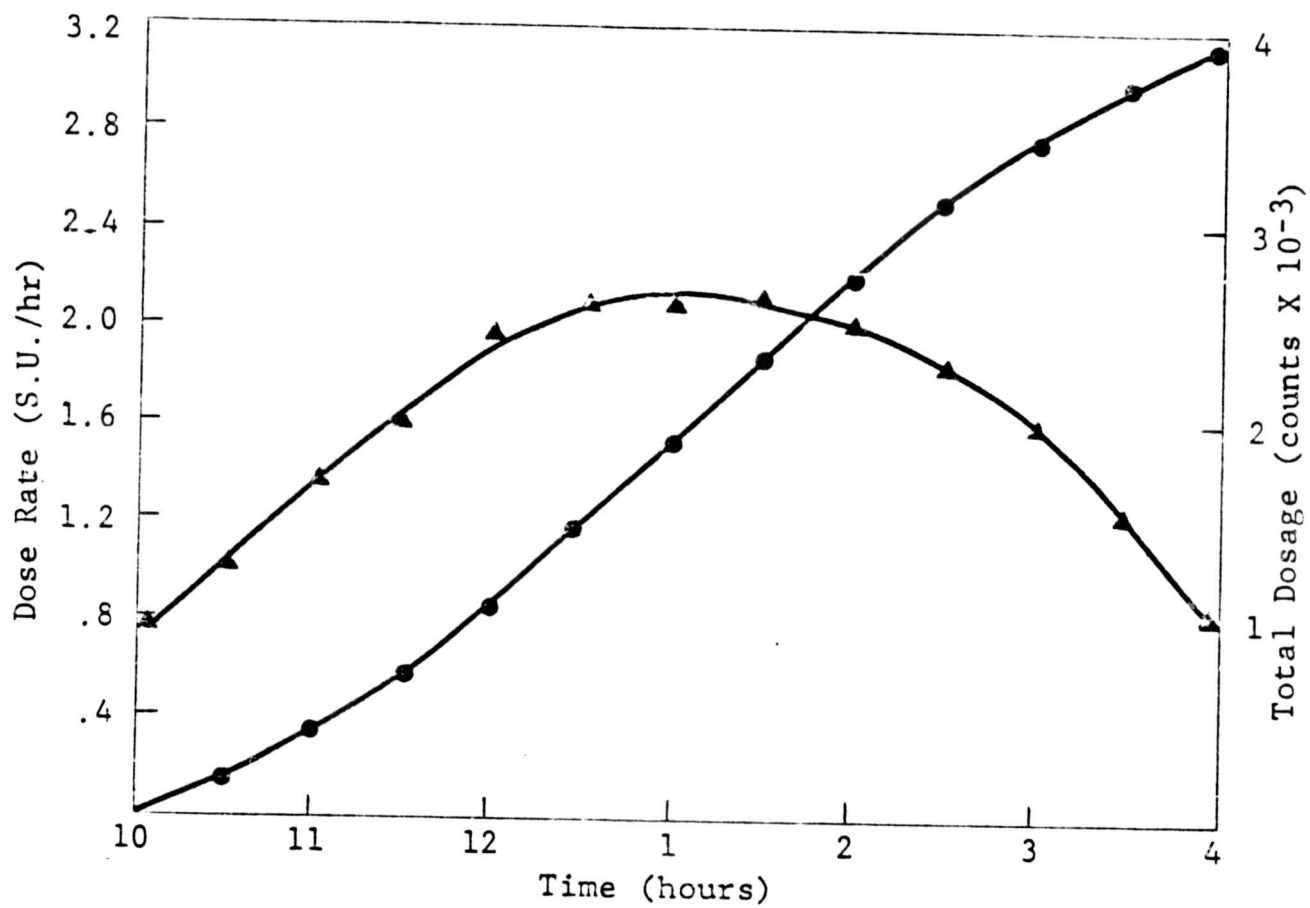


Figure 11. Ambient UV-B conditions at the air/water interface during August, 1978.

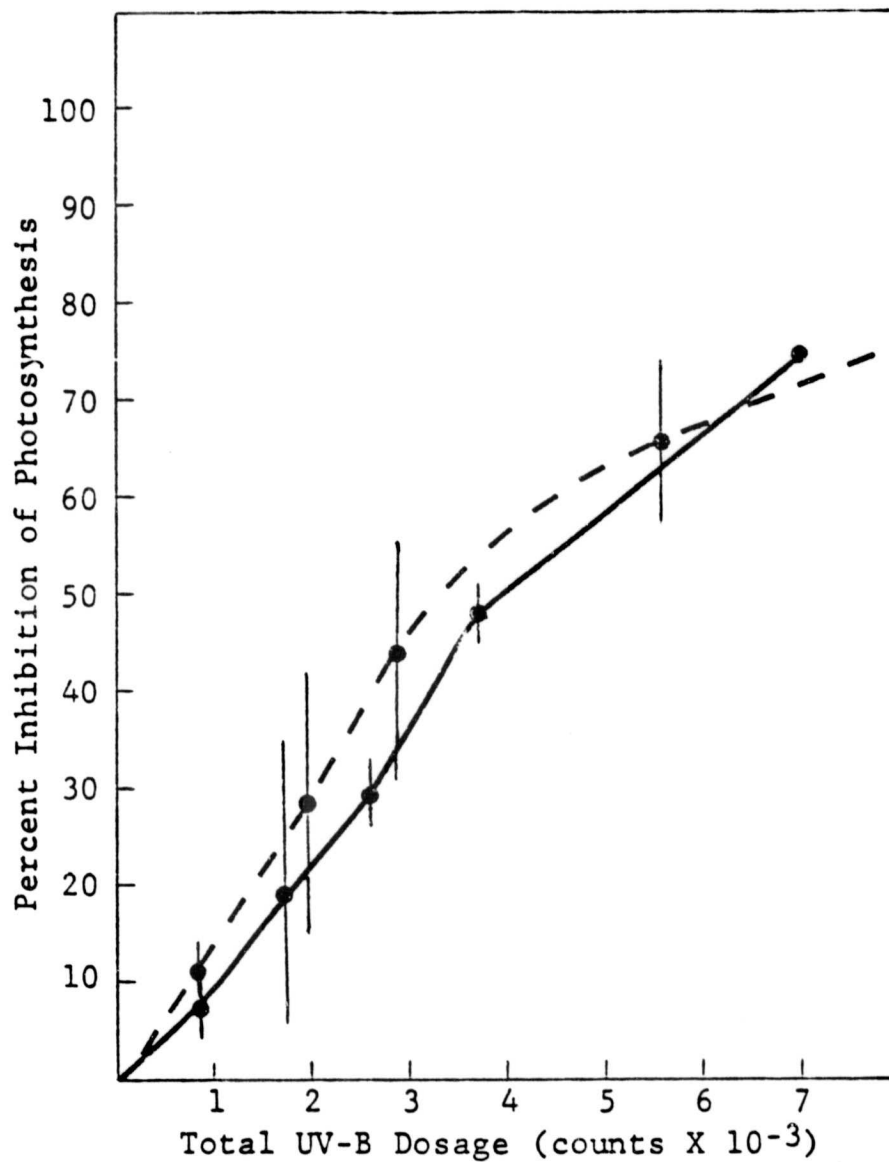


Figure 12. Photosynthetic response of Halophila engelmannii to UV-B in the presence of 700 $\mu\text{E}/\text{m}^2/\text{sec}$ PAR; UV-B and PAR, (●—●); UV-B alone, (●--●); vertical lines, standard deviation; closed circles, mean.

encountered may be more costly than the photosynthetic inhibition produced during periods of exposure.

At first glance the response of Syringodium to UV-B in the presence of PAR (fig. 13) would seem to indicate some attenuation of photosynthetic inhibition. However, this was not the case as UV-B alone produced far less photosynthetic inhibition than UV-B and PAR combined. The increased inhibition was obtained with every UV-B dose rate provided. This apparent contradiction is discussed in section D.

Halodule was the only seagrass to give evidence of a significant photorepair mechanism. All UV-B dose rates supplied were tolerated well with $700 \mu\text{E}/\text{m}^2/\text{sec}$ backlighting (the distinction between dose rates was lost), limiting the amount of photosynthetic inhibition to about 10 percent (fig. 14). This held true up to a total UV-B dosage of 5500 counts, almost triple the normal dosage encountered. It appears PAR had no noticeable effect at dose rates of less than 16 CPM. Photorepair at dose rates of 12-16 CPM didn't become apparent until approximately 5000 counts was reached; at lower dose rates it was never visible. Photorepair responded most clearly to the higher dose rates. Interestingly enough, the addition of PAR to the experimental system appeared to be inhibitory at low levels of UV-B exposure with Halodule (as it was with Syringodium).

D) The UV-A effect.

A group of experiments performed with Halophila has provided one possible explanation for the apparent inhibitory nature of the UV-B, PAR combination. The photorepair capabilities of Halophila were not only

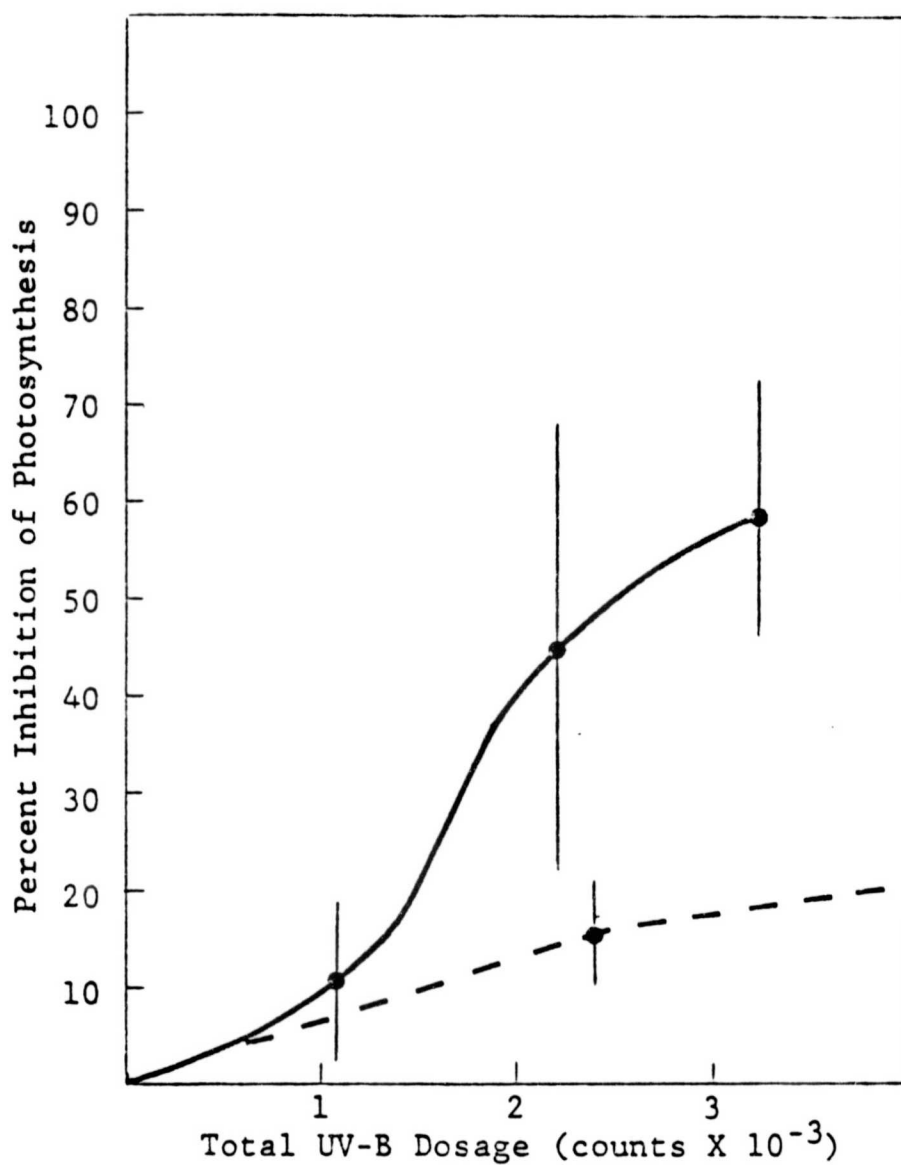


Figure 13. Photosynthetic response of Syringodium filiforme to UV-B in the presence of 700 $\mu\text{E}/\text{m}^2/\text{sec}$ PAR; UV-B and PAR, (●—●); UV-B alone, (●--●); vertical lines, standard deviation; closed circles, mean.

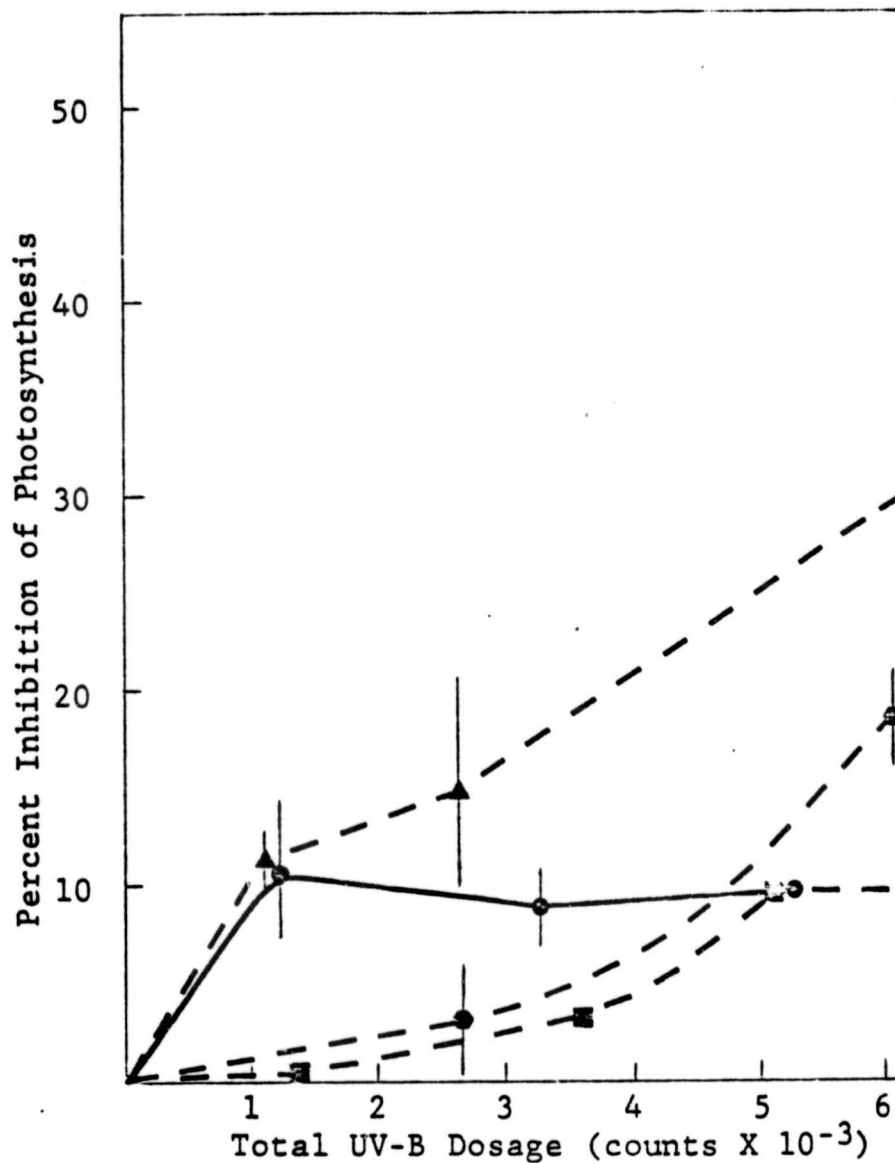


Figure 14. Photosynthetic response of *Halodule wrightii* to UV-B in the presence of 700 $\mu\text{E}/\text{m}^2/\text{sec}$ PAR; UV-B and PAR, (●—●); UV-B alone, (●--●); dose rates: 18-20 CPM, (▲--▲); 12-16 CPM, (●--●); 6-8 CPM, (■--■); vertical lines, standard deviation, closed points, mean.

tested at different dose rates of UV-B, but also several different PAR intensities: 0, 250, 500, and 700 $\mu\text{E}/\text{m}^2/\text{sec}$. Evidence obtained from these experiments suggests UV-A is a potent inhibitor of photosynthesis when combined with certain PAR intensities. It is important to point out here that control samples under Mylar are not completely protected from ultraviolet radiation; ultraviolet-A (315-400 nm) is capable of penetrating this filter. Therefore the actual experimental conditions were such that the controls received UV-A and PAR exposures, while the test samples were exposed to UV-A and UV-B in addition to PAR.

With the addition of PAR at low intensities to ultraviolet treatments extensive photosynthetic inhibition was found in control samples, as well as the test samples. This was totally unexpected. Subsequent experiments showed a similar inhibitory effect at other PAR intensities. Figure 15 shows the effects of UV-A on photosynthesis by Halophila at different PAR intensities. One important constraint on this study was the lack of a spectral radiometer to accurately measure the amount of UV-A experienced by the leaf samples. The Sunburn Ultraviolet Meter used does not respond to UV-A, therefore the UV-A effect had to be characterized in terms of the accompanying UV-B radiation. The UV-A effect was monitored at a UV-B dosage of 2000 counts, at UV-B dose rates of 11 and 18 CPM. In addition to the Mylar covered samples, tissues were also placed beneath Kodacel and exposed simultaneously to the UV-A,B, and PAR conditions. These Kodacel samples provided a measure of photosynthetic inhibition produced by the combination of UV-A and UV-B at each PAR intensity (fig. 16). Perhaps the most important information gained from these experiments was that photosynthesis must be proceeding,

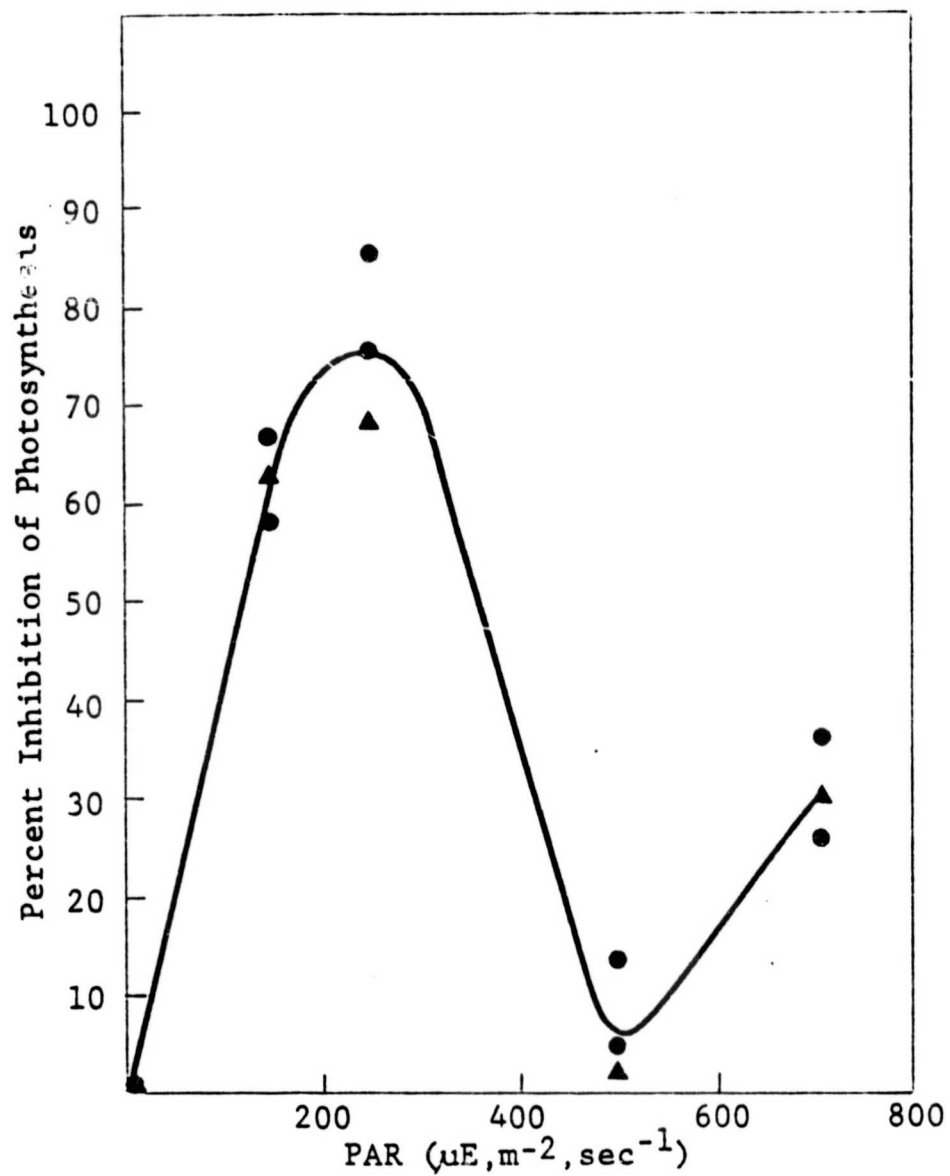


Figure 15. Effects of UV-A on photosynthesis by Halophila engelmannii as a function of PAR intensity; UV-B dosage, 2000 counts; UV-B dose rates: 11 CPM, ● ; 18 CPM, ▲ .

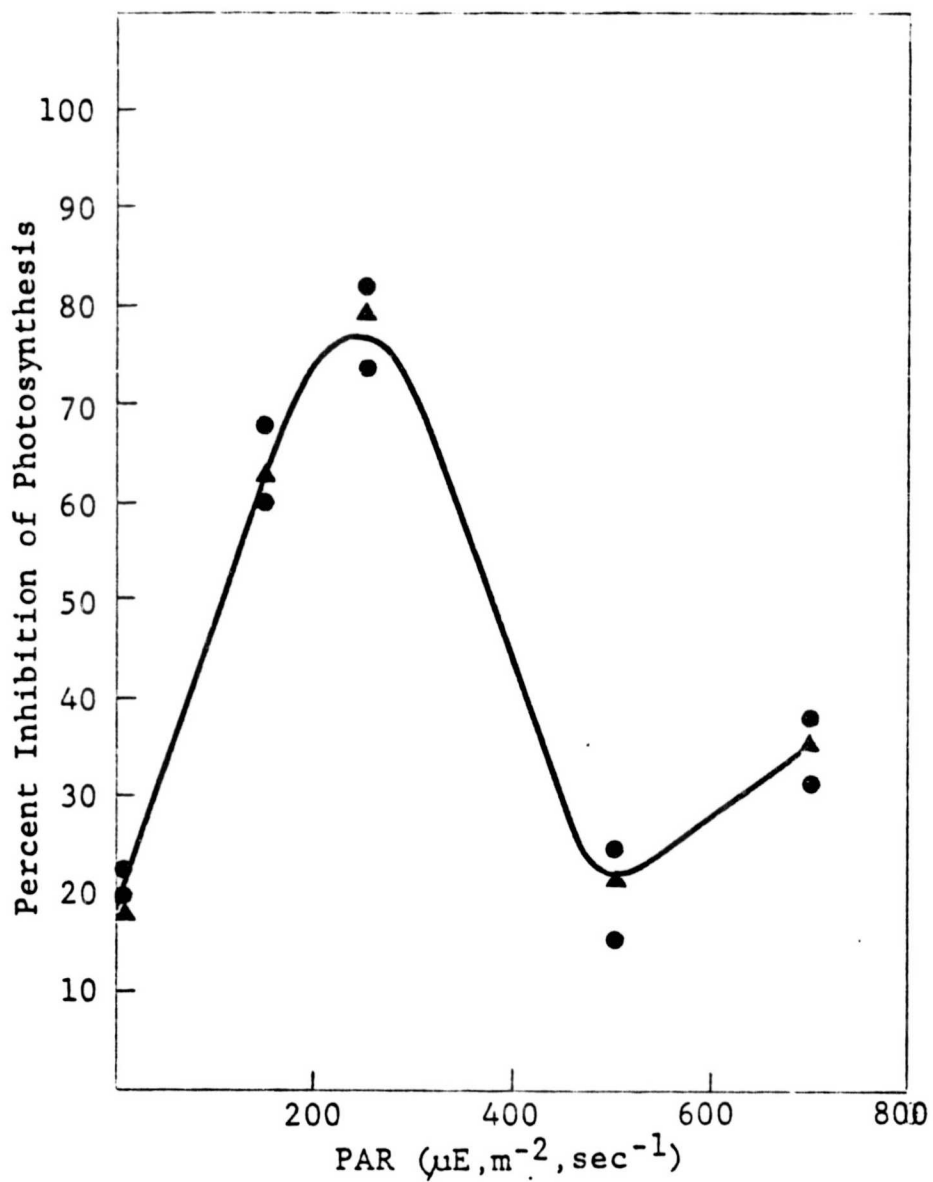


Figure 16. Effects of UV-A and UV-B on photosynthesis by Halophila engelmannii as a function of PAR intensity; UV-B dosage, 2000 counts; UV-B dose rates: 11 CPM, ● ; 18 CPM, ▲ .

at least minimally, for the UV-A effect to be observed. Control samples receiving UV-A in the absence of PAR were unaffected. A comparison of photosynthetic inhibition in Halophila as a function of PAR intensity due to UV-A and the combination of UV-A and UV-B is found on figure 17. In this seagrass the UV-A effect was most damaging at a PAR intensity of 250 $\mu\text{E}/\text{m}^2/\text{sec}$. At 500 $\mu\text{E}/\text{m}^2/\text{sec}$ the inhibition of photosynthesis in the presence of UV-A was the lowest observed save for the complete absence of PAR. This PAR intensity apparently prevents or repairs damage induced by UV-A. When a PAR intensity of 700 $\mu\text{E}/\text{m}^2/\text{sec}$ was applied the UV-A effect again became evident. It is possible photooxidation or solarization may have occurred at this intensity due to the presence of UV-A, and 700 $\mu\text{E}/\text{m}^2/\text{sec}$ alone failed to cause such a response in preliminary experiments of photosynthetic rates. The lack of data characterizing the UV-A component or ambient ultraviolet radiation in the natural system prevents the resolution of this question. In portions of the Indian River where Halophila is abundant, light intensities of 500 $\mu\text{E}/\text{m}^2/\text{sec}$ are more common than those approximating 700 $\mu\text{E}/\text{m}^2/\text{sec}$ and may indicate an adaptive response to the lower light intensity to reduce effects of UV-A. It is possible that the unexpected results obtained in the photorepair studies of Halodule and Syringodium may also be attributable to UV-A irradiation.

E) In situ experiments.

Laboratory studies of UV-B irradiation were augmented by a series of in situ experiments to determine if the seagrasses were currently being inhibited by natural levels of UV-B in the presence of ambient PAR. Field incorporation chambers were placed at the mean leaf depth for each sea-

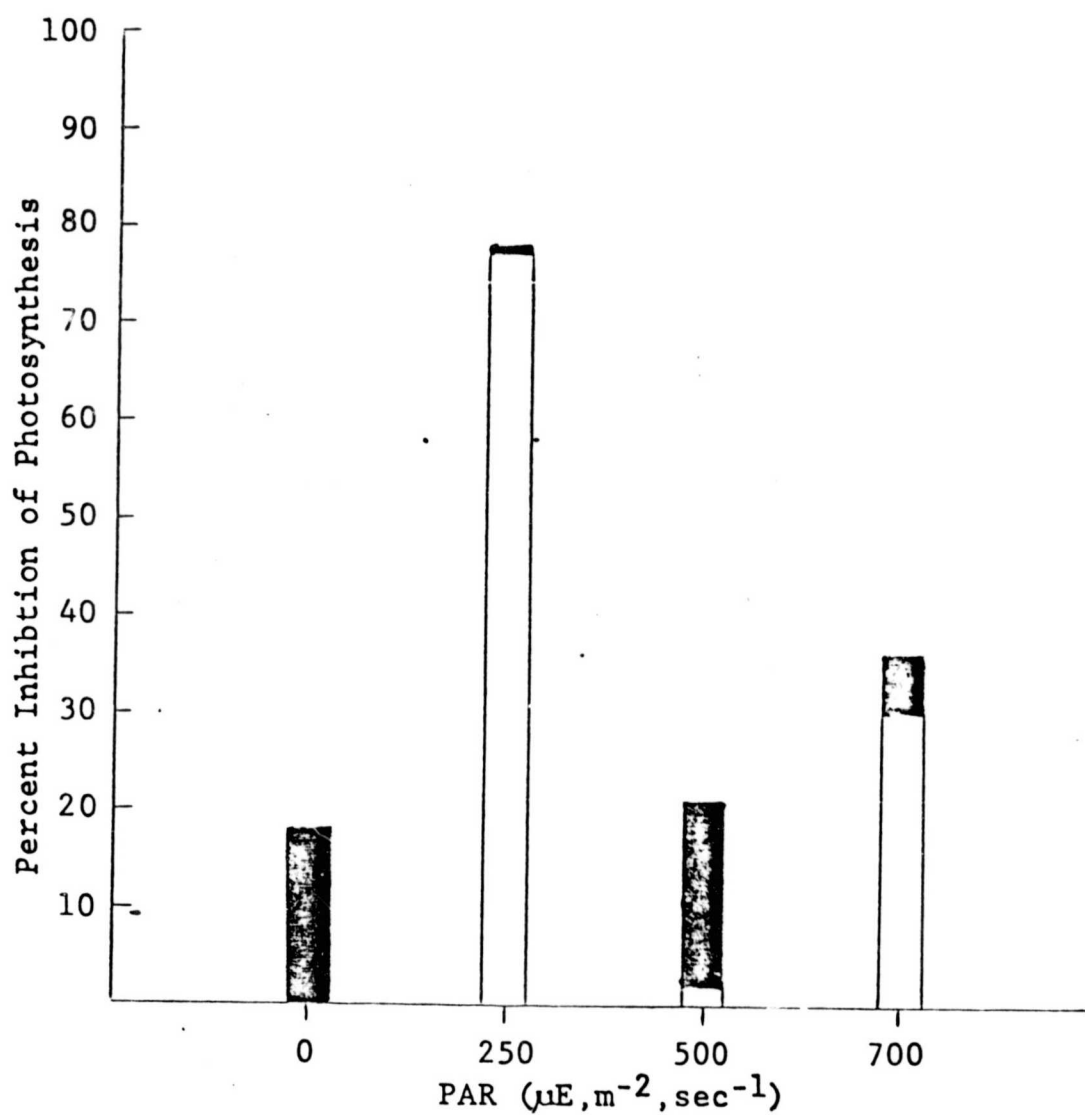


Figure 17. Comparison of UV-A and UV-B effects on photosynthesis by Halophila engelmannii as a function of PAR intensity; UV-A effect, $\square$; UV-B effect $\blacksquare$.

grass for these experiments. Results are inconclusive at this point. In all cases there was no apparent photosynthetic inhibition due to UV-B, but the possibility remains that UV-A (which could not be measured) was inhibiting the Mylar controls so it would only "appear" as though no photosynthetic inhibition occurred. However this could just as easily be explained on the basis of aged Mylar's reduced transmission of PAR (the nature of PAR exposure in the laboratory circumvents this problem) in comparison to Kodacel. Further investigation is necessary to answer these questions and determine the true environmental significance of UV-A and UV-B.

F) Epiphyte studies.

The possible attenuation of UV-B induced photosynthetic inhibition by epiphytic growth upon the seagrasses was examined. The results of these experiments are illustrated in figure 18. It seems likely that the physical blocking of UV-B and the resultant reduction in photosynthetic inhibition is a coincidence the seagrasses take advantage of, but is by no means the principle method the seagrasses rely upon to reduce UV-B damage. Previous work from this laboratory has monitored the fluctuation in epiphyte populations throughout the year (fig. 19). These boom and bust cycles do not follow the elevation of the sun through its yearly course and reliance upon such a discontinuous method of UV-B protection could be very costly. Be this as it may, the presence of thick epiphytic layers upon leaf surfaces did indeed reduce the amount of UV-B induced photosynthetic inhibition in comparison to denuded tissues. Whether this is of any great importance to the seagrass in terms of overall metabolic

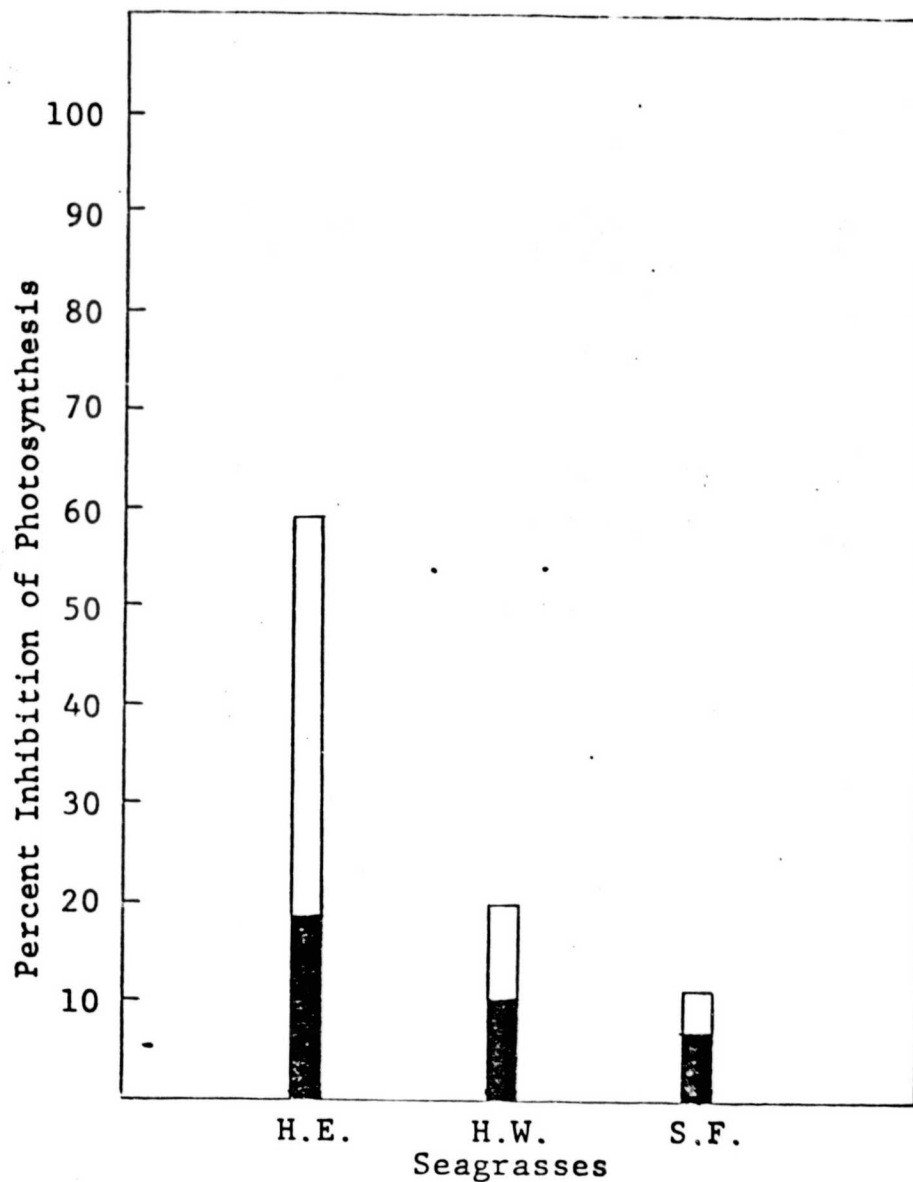


Figure 18. Attenuation of photosynthetic inhibition by epiphytic shielding; UV-B dose rate, 16 CPM, epiphytic growth (mg epiphyte/gm leaf tissue): Halophila engelmannii, 100.27 mg; Halodule wrightii, 22.15 mg; Syringodium filiforme, 75.47 mg; epiphytes present, ■ ; epiphytes removed, □ .

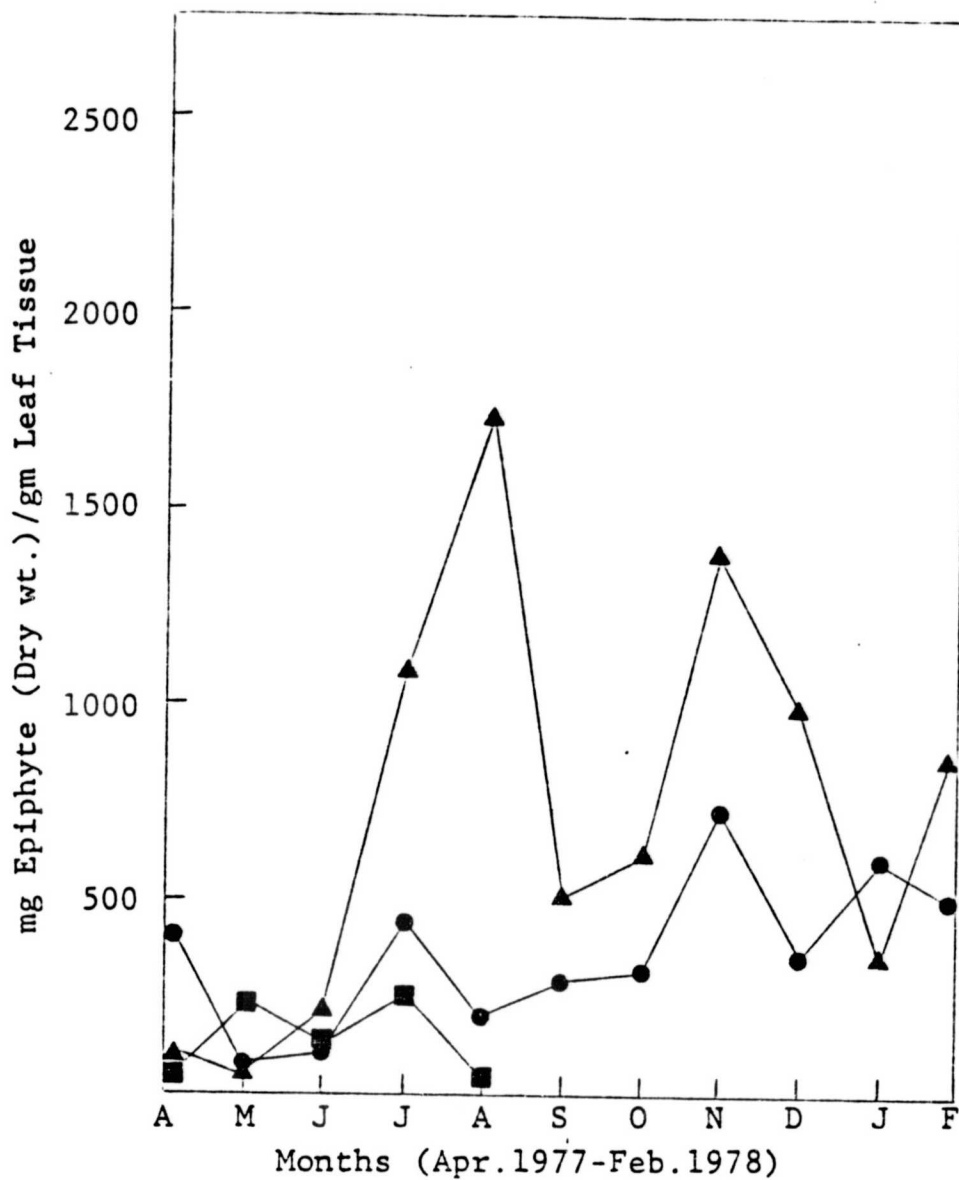


Figure 19. Epiphyte abundance from April 1977 to February 1978; Halophila engelmannii, (■—■); Halodule wrightii, (▲—▲); Syringodium filiforme, (●—●).

output is questionable; this "shield" would also reduce the penetration of PAR to some degree and under suboptimal conditions reduce the photosynthetic rate of the underlying tissue. Halodule and Syringodium often take on a cattail-like appearance at the height of the epiphytic blooms, which may be of particular value when the leaves approach the air/water interface. Halophila as a general rule is sparsely covered with epiphytes as best. This may be due to its location in the lower portion of the photic zone. Photosynthetic epiphytes may not be able to maintain themselves under such low light conditions.

Conclusions

The impact of UV-B appears to be minimal at this time in regard to the seagrasses of the Indian River lagoonal system. Halophila engelmanni, while it has no apparent intrinsic tolerance to UV-B, is protected by the depth and/or turbidity of the waters where it is abundant. Syringodium filiforme also seems to lack efficient repair mechanisms; morphological adaptation (a thick epidermal cell layer and concentration of photosynthetic tissue in the leaf core) apparently reduces the rate of UV-B induced photosynthetic damage to tolerable levels. Halodule wrightii was the only seagrass studied which had both an intrinsic tolerance to UV-B and an effective photorepair mechanism to reverse UV-B induced damage. In the final analysis these seagrasses, either of themselves, through aquatic absorption of UV-B, or occasional epiphytic shielding, appear to be relatively safe from UV-B increases save those due to extreme atmospheric ozone depletion.

This study however has raised the question as to whether UV-B or UV-A is of greater consequence in the biosphere. At this point three statements may be made concerning UV-A. First, the UV-A effect is real and repeatable in Halophila engelmannii. Empirical evidence exists suggesting the UV-A is also damaging to Halodule wrightii and Syringodium filiforme, but specific experimentation is lacking. Second, photosynthesis must be operating, as least minimally, for UV-A to have a measurable inhibitory effect. Finally, the UV-A effect apparently changes as a function of PAR intensity. The characterization of the UV-A effect may have a significant impact on the understanding of seagrass distribution and productivity.

Literature Cited

1. Basiouny, F. M., T. K. Van, and R. H. Biggs. 1978. Some morphological and biochemical characteristics of C₃ and C₄ plants irradiated with UV-B. *Physiol. Plant.* 42:29-32.
2. Biggs, R. H., D. E. Brabham, W. F. Campbell, L. A. Garrard, J. R. Brandle, W. B. Sisson, and M. M. Caldwell. 1975. Plant Responses to UV Radiation. Climatic Impact Assessment Program. U.S. Dept. of Transportation, Report No. DCT-TST-75-55. pp. 4-1 to 4-58.
3. Brandle, J. R., W. F. Campbell, W. B. Sisson, and M. M. Caldwell. 1977. Net photosynthesis, electron transport capacity, and ultrastructure of Pisum sativum L. exposed to ultraviolet-B radiation. *Plant Physiol.* 60:165-169.
4. Calkins, J. 1975. Effects of real and simulated solar UV-B radiation in a variety of aquatic microorganisms - possible implications for aquatic ecosystems. Climatic Impact Assessment Program, U.S. Dept. of Transportation, Report No. DCT-TST-75-55. pp. 5-31 to 5-71.
5. Mantai, K. E., J. Wong, and N. I. Bishop. 1970. Comparison studies on the effects of ultraviolet irradiation on photosynthesis. *Biochem. Biophys. Acta.* 197:257-366.
6. McKnight, G. and D. S. Nachtwey. 1975. Natural resistance of freshwater algae to UV radiation - a survey. Climatic Impact Assessment Program. U.S. Dept. of Transportation, Report No. DCT-TST-75-55. pp. 5-73 to 5-79.

7. Murphy, T. M. and D. S. Nachtwey. 1975. General Aspects of UV Radiation Effects on Biological Systems. Climatic Impact Assessment Program. U.S. Dept. of Transportation, Report No. DCT-TST-75-55. pp. 3-1 to 3-84.
8. Nachtwey, D. S. 1976. Potential effects on aquatic ecosystems of increased UV-B radiation. In: Proceedings of the Fourth Conference on the Climatic Impact Assessment Program. U.S. Dept. of Transportation, Report No. DCT-TST-75-38.
9. Nachtwey, D. S., M. M. Caldwell, and R. H. Biggs, eds. Impacts of Ultraviolet Radiation. Monograph V. Climatic Impact Assessment Program. U.S. Dept. of Transportation, Report No. DCT-TST-87-55.
10. Phillips, R. C. 1960. Observations on the ecology and distribution of the Florida seagrasses. Prof. Pap. Ser. No. 2 Fla. St. Bd. Conserv. Mar. Lab. St. Petersburg, Fla.
11. Sisson, W. B. and M. M. Caldwell. 1976. Photosynthesis, dark respiration, and growth of Rumex patientia L. exposed to ultraviolet irradiance (288 to 315 nanometers) simulating a reduced atmospheric ozone column. Plant Physiol. 58:563-568.
12. Strain, H. and W. A. Svec. 1966. Extraction, separation, estimation, and isolation of the chlorophylls. In: L. P. Vernon and G. R. Sheely, eds. The Chlorophylls. Academic Press, New York. pp. 21-66.
13. Strickland, J. D. and T. R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Queens Printer, Ottawa. pp. 27-34.
14. Taras, M. J., A. E. Greenberg, R. D. Hoak, and M. C. Rand, eds. 1971. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, New York. pp. 474-481.

15. Thai, V. K. and L. A. Garrard. 1975. Effects of UV-B radiation on the net photosynthesis and the rates of partial photosynthetic reactions of some crop plants. Climatic Impact Assessment Program. U.S. Dept. of Transportation, Report No. DCT-TST-75-55. pp. 4-125 to 4-145.
16. Wells, G. N. and D. S. Nachtwey. 1978. The effects of ultraviolet irradiation on photosynthetic carbon reduction by Ruppia maritima L. Photochem. Photobiol. (in press).